Effects of low-moisture, sugarcane molasses-based block supplementation on growth, physiological parameters, and liver trace mineral status of growing beef heifers fed low-quality, warm-season forage

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ABSTRACT: The objectives of the study were to evaluate the growth, physiological parameters, and liver trace mineral status of beef heifers provided low-quality warm-season forage and different forms (meal vs. block) of trace mineral-fortified supplementation. One hundred yearling Nellore heifers were blocked by initial body weight (BW) (184 ± 2.5 kg) and randomly assigned into 1 of 20 drylot pens (5 heifers/pen). Treatments were randomly assigned to pens (5 pens/treatment) and consisted of heifers receiving: 1) a loose meal trace mineral supplement (TM; De Heus Animal Nutrition Industry); 2) free choice access to a low-moisture, cooked sugarcane molasses-based protein block (LMB); 3) isocaloric and isonitrogenous, loose meal protein supplement pair-fed to LMB supplement dry matter (DM) intake (PSPF); and (4) loose meal protein supplement offered at 0.2% of BW (PS). Supplements were formulated to achieve same daily intake of supplemental trace mineral among treatments. Hence, TM supplement was offered at 66.6% of the supplement DMI of LMB heifers. Heifers were offered free choice access to water and ground brachiaria (*Brachiaria brizantha*) hay from day 0 to 45. Overall average daily gain from day 0 to 45 was the least for TM heifers ($P \leq 0.05$) and did not differ among LMB, PSPF, and PS heifers ($P \geq 0.60$). Daily hay DMI did not differ among treatments ($P \geq 0.63$). Total intake of DM and TDN were least for TM heifers ($P \leq 0.03$) and did not differ ($P \geq 0.66$) among LMB, PSPF, and PS heifers. Total supplemental intake of crude protein (CP) and rumen degradable protein (RDP) and total intake of CP and RDP (supplement + hay) were least for TM and greatest for PS heifers ($P \leq 0.05$), and intermediate for LMB and PSPF heifers ($P \geq 0.70$). Effects of treatment × day and treatment were not detected ($P \geq 0.61$) for plasma concentrations of insulin-like growth factor 1 (IGF-1), and non-esterified fatty acids (NEFA). Effects of treatment were detected for plasma concentrations of PUN ($P = 0.005$) and tended to be detected for plasma concentrations of glucose ($P = 0.08$), which were least for TM heifers ($P \leq 0.03$) and did not differ ($P \geq 0.17$) among LMB, PSPF, and PS heifers. Trace mineral intake and liver concentrations of all trace minerals did not differ ($P \geq 0.13$) among treatments. Hence, the use of LMB supplementation resulted in positive effects on growth without impacting trace mineral status compared to a loose meal trace mineral salt, and similar growth performance and trace mineral status compared to a conventional protein supplementation offered at 0.2% of body weight.

Key words: block, heifers, molasses, Nellore, protein supplement, trace mineral

INTRODUCTION

Low-moisture, cooked molasses blocks (LMB) for forage-fed cattle is a popular supplementation strategy due to its convenience, decreased production costs (labor and fuel), and potential for improving forage intake and digestion (Löest et al., 2001) and grazing of underutilized pastures (Bailey and Welling, 1999). The improved forage digestion can be attributed to the supply of RDP, which is often the most limiting nutrient under grazing of low-quality grasses (Köster et al., 1996; Titgemeyer et al., 2004). It is also possible to use mineral-fortified LMB as an efficient strategy to improve the trace mineral status of beef calves (Ranches et al., 2018). For instance, beef calves grazing bahiagrass pastures and fed trace mineral-fortified LMB had greater liver concentrations of Co, Cu, Mn, Se, and Zn compared with a nonfortified LMB, despite the less supplement dry matter intake (DMI) of fortified vs. nonfortified LMB calves (Ranches et al., 2018).

The manufacturing process of LMB, particularly extreme heat or pH, may alter the bioavailability of nutrients, such as ammonia release in the rumen (Trater et al., 2003; Katulski et al., 2017), whereas the delivery form of supplements (loose meal vs. block form) may alter supplement consumption patterns and nutrient utilization (Katulski et al., 2017). Nevertheless, LMB supplementation for growing beef heifers fed low-quality forage may lead to improved nutrient consumption and trace mineral status, and consequently growth, compared with trace mineral salt offered in loose meal form. In addition, due to improved nutrient utilization, LMB supplementation may lead to similar performance compared to conventional supplementation strategies offering greater amounts of protein supplements. The objectives of the study were to evaluate growth, physiological parameters, and liver trace mineral status of beef heifers fed low-quality forage and offered different forms (meal vs. block) of trace mineral-fortified supplementation.

MATERIALS AND METHODS

Animals and Experiment Design

The study described herein was conducted at São Paulo State University (São Manuel, São Paulo, Brazil) from November to December 2017. All animals were cared for by acceptable practices as outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010) and approved by the São Paulo State University Animal Care and Use Committee.

One hundred Nellore heifers were stratified and blocked by initial BW (184 ± 2.5 kg; 12–13 mo of age), and then randomly assigned into 1 of 20 drylot pens (5 blocks; 4 pens/block; 200 m² and 5 heifers/pen). Treatments were randomly assigned to pens within each block (one pen/treatment/block; five pens/treatment), and consisted of heifers receiving: 1) a complete trace mineral mix supplement offered in a loose meal form (TM; De Heus Animal Nutrition Industry, Rio Claro, São Paulo); 2) free choice access to a low-moisture, cooked sugarcane molasses-based protein block (LMB; MUB, De Heus MBU Brazil Animal Nutrition Industry, Guararapes, São Paulo); 3) protein supplement offered in a loose meal form and pair-fed to achieve isocaloric and isonitrogenous supplement intake compared to LMB heifers (PSPF); and 4) a commercial protein supplement offered in a loose meal form and at levels recommended by manufacturer (PS; DM basis; De Heus Animal Nutrition Industry, Rio Claro, Sao Paulo). Treatments were offered from day 0 to 45 and all supplements included the same vitamin/trace mineral premix (dry matter [DM] basis: 3% Ca, 10% Mg, 23.5% S, 600 mg/kg Co, 20,000 mg/kg Cu, 264 mg/kg Se, 1,200 mg/kg I, 80,000 mg/kg Zn, 53,200 mg/kg Mn, 4,000,000 mg/kg vitamin A, 400,000 mg/kg vitamin D_3, 20,000 mg/kg vitamin E, and 40,000 mg/kg monensin, Poulcox 40, Peshteria, Bulgaria). All supplements were formulated to achieve same daily supplemental trace mineral intake among treatments. Hence, within each block of pens: 1) TM supplement was offered daily at 66.6% (DM basis) of the supplement DMI of LMB heifers obtained in the previous day; 2) PSPF supplement DM offered was adjusted daily to achieve similar daily intake of supplemental TDN and CP compared to LMB heifers; and 3) PS supplement was offered at 0.2% of heifer initial BW (DM basis), which is the manufacturer recommended level offered in commercial beef cattle operations. Supplement DM offered to PS heifers was adjusted accordingly to the average BW obtained on day 24 and 25. Each pen assigned to LMB treatment received a single 50-kg supplement block from day 0 to 45, but each LMB block was weighed daily at 0730 h to calculate supplement DMI from previous day. Rainfall precipitation was observed on 2 d, and on these days, supplement DMI was not calculated and removed from statistical analyses. All remaining supplements were hand-fed daily at 0800 h from day 0 to 45. Heifers were offered free choice access to water and ground brachiaria (*Brachiaria brizantha*) hay from day 0 to 45. Hay was chopped to achieve between 2 and

Table 1. Average chemical composition of supplements provided from day 0 to 45

| Item[2] | Supplement[1] | | | |
	TM	LMB	PSPF	PS
Ingredients, % (as-fed basis)				
Dried sugarcane molasses	-	53.0	-	-
Ground corn	-	-	54.2	45.0
Cottonseed meal	-	14.3	10.3	-
Soybean meal	-	-	-	16.0
Urea	-	13.0	13.0	5.0
Kaolin[3]	-	-	4.6	3.5
Limestone	40.5	-	0.80	10.8
Ca phosphate	15.0	8.7	8.8	4.0
NaCl	40.0	5.0	5.0	15.0
Trace mineral/vitamin premix[4]	3.8	2.5	2.5	0.63
Mg oxide	0.67	0.79	0.80	0.25
Soybean oil	-	2.7	-	-
Palatability enhancer[5]	0.05	-	-	-
		--- DM basis ---		
TDN[6], %	-	50.7	50.7	53.3
CP, %	-	46.0	46.0	25.0
RDP, % of CP	-	93.2	95.5	83.6
ADF, %	-	7.5	9.1	9.9
Ca, %	18.6	2.3	2.3	5.0
P, %	3.0	2.0	2.0	1.0
Mg, %	1.3	0.93	0.93	1.8
K, %	0.03	2.41	0.30	0.32
Na, %	21.0	2.0	1.9	4.7
S, %	1.7	0.86	0.74	0.95
Cu, mg/kg	712	606	612	121
Fe, mg/kg	3214	2179	2088	435
Mn, mg/kg	3738	2467	2299	459
Se, mg/kg	119	79	81	15
Zn, mg/kg	2864	1907	1982	389

[1]TM = a complete vitamin/trace mineral mix supplement offered in a loose meal form (66.6% of LMB supplement DMI); LMB = free choice access to a low-moisture, cooked sugarcane molasses-based block; PSPF = protein supplement offered in a loose meal form and pair-fed to achieve similar supplement intake of DM, TDN, and CP of LMB heifers; PS = a commercial protein supplement offered in a loose meal form at 0.2% of BW (DM basis).

[2]Samples of supplements were collected daily and pooled within each week, and then sent in duplicate to a commercial laboratory (3rlab) for wet chemistry analysis of all nutrients.

[3]Rumen-inert indigestible substance included.

[4]DM basis: 3% Ca, 10% Mg, 23.5% S, 600 mg/kg Co, 20,000 mg/kg Cu, 264 mg/kg Se, 1,200 mg/kg I, 80,000 mg/kg Zn, 53,200 mg/kg Mn, 4,000,000 mg/kg vitamin A, 400,000 mg/kg vitamin D$_3$, 20,000 mg/kg vitamin E, and 40,000 mg/kg monensin, Poulcox 40, Peshteria, Bulgaria).

[5]Tecnaroma zta sweet note fruit red 4W/10638 powder (New Products Comercial Agricola e Veterinária, Campinas, São Paulo, Brazil).

[6]Calculated as described by Weiss et al. (1992).

5 cm of length. Hay and supplements were offered in separated feed bunks. Chemical composition of hay and supplements are shown in Table 1.

Data Collection

Except for LMB, supplements were consumed entirely within 1 h after feeding. Hay DM offered and refused were obtained daily for each pen by drying samples of hay offered and refused in a forced-air oven at 56 °C for 48 h. Daily DMI of LMB supplement was estimated by multiplying the daily DM concentration of the supplement by the weight disappearance of each supplement block obtained between consecutive days. Daily total DMI was determined by subtracting the daily hay DM refused from the total daily hay and supplement DM offered. Samples of supplement offered were collected daily and pooled within each week, and then sent in duplicate to a commercial laboratory (3rlab, Lavras, Minas Gerais, Brazil) for wet chemistry analysis of all nutrients (Table 1). Samples of hay offered were collected daily and pooled within every 15 d, and then sent in duplicate

Table 2. Average chemical composition of bahia-grass hay offered from day 0 to 15, 16 to 30, and 31 to 45

Item	Hay[1]		
	Day 0 to 15	Day 16 to 30	Day 31 to 45
	---------------------- DM basis ----------------------		
TDN, %	42.5	45.6	60.2
CP, %	5.8	4.9	6.6
RDP, % of CP	83.7	84.5	86.0
ADF, %	47.9	49.4	41.6
Ca, %	0.21	0.22	0.33
P, %	0.11	0.10	0.08
Mg, %	0.25	0.21	0.22
K, %	0.33	0.21	0.85
Na, %	0.04	0.06	0.12
S, %	0.28	0.24	0.15
Cu, mg/kg	5.5	5.0	6.4
Fe, mg/kg	216	391	179
Mn, mg/kg	93	88	119
Se, mg/kg	0.10	0.08	0.10
Zn, mg/kg	30	32	41

[1]Samples of hay were collected daily and pooled within each 15-d period, and then sent in duplicate to a commercial laboratory (3rlab) for wet chemistry analysis of all nutrients.

to a commercial laboratory (3rlab) for wet chemistry analysis of all nutrients (Table 2). Samples were analyzed for concentrations of CP (method 984.13; AOAC, 2006), and acid detergent fiber (ADF) (method 973.18 modified for use in an Ankom 200 fiber analyzer; Ankom Technology Corp., Fairport, NY; AOAC, 2006). Concentrations of TDN were calculated as proposed by Weiss et al. (1992).

Individual full BW of heifers were assessed at 0730 h on 2 consecutive days (day 0 and 1, 24 and 25, and 45 and 46), immediately before morning feeding. Shrunk BW were not obtained during the study to avoid shrink-induced stress effects on forage and supplement DMI and blood physiological parameters that could interfere with data interpretation. Blood samples (10 mL) were collected from jugular vein on day 0, 24, and 45, immediately before feeding into sodium-heparin (158 USP) containing tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ), placed on ice immediately after collection, and then centrifuged at 1,200 × *g* for 25 min at 4 °C. Plasma was stored frozen at −20 °C until later laboratory analyses.

On day 0, three heifers from each pen were randomly selected and assigned to liver tissue biopsies on day 0 and 45. Liver samples (100 mg of tissue wet weight) were collected via needle biopsy following the procedure described by Arthington and Corah (1995), and then stored at −20 °C. Samples were then assessed for trace mineral concentrations at Michigan State University Diagnostic Center for Population & Animal Health (Lansing, MI). Liver trace mineral concentrations on day 0 were initially included as covariate to adjust liver trace mineral concentrations on day 45, but later removed from statistical model ($P \geq 0.22$). Liver samples were collected only on day 0 and 45: 1) because our goal was to evaluate the final liver trace mineral concentrations of heifers after receiving their respective supplement for 45 d, and 2) to avoid a surgery-induced inflammatory response in the middle term of the study that could interfere with growth performance and physiological parameters. Average total trace mineral consumption from day 0 to 45 was calculated by multiplying the total DMI of hay and supplement by the average weekly mean concentration of each trace mineral present in hay and supplement.

Laboratory Analyses

Plasma concentrations of insulin were determined using a single chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Intra- and interassay coefficient of variation (CV) for insulin were 1.9% and 2.8%, respectively. Commercial quantitative colorimetric kits were used to determine the plasma concentrations of glucose (G7521; Pointe Scientific, Inc., Canton, MI), PUN (B7551; Pointe Scientific Inc., Canton, MI), and NEFA (HR Series NEA-2; Wako Pure Chemical Industries Ltd. USA, Richmond, VA). Inter- and intra-assay CV for assays of glucose, PUN, and NEFA were 2.7% and 3.4%, 3.2% and 5.8%, and 3.9 and 4.2%, respectively. Plasma IGF-1 concentrations were analyzed in duplicate samples using commercial enzyme-linked immunosorbent assay kits (SG100; R&D Systems Inc., Minneapolis, MN) previously validated for bovine samples (Moriel et al., 2012). Inter- and intra-assay CV for IGF-1 assay were 1.81% and 2.35%, respectively.

Statistical Analyses

All data were analyzed as randomized complete block design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, version 9.4) with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. Pen was the experimental unit, whereas pen(treatment) and heifer(pen) were included as random effects in all analyses. Heifer BW, blood parameters, liver concentrations of trace minerals, and daily DMI of hay and supplement were

analyzed as repeated measures, and tested for fixed effects of treatment, time, and resulting interaction, using pen(treatment) as the subject. The covariance structure was chosen using the lowest Akaike information criterion. Compound symmetry was used as the covariance structure in all statistical analyses, except for statistical analyses of plasma concentrations of glucose, PUN, and NEFA that used the autoregressive 1 covariance structure. Heifer BW on day 0 and plasma concentrations of glucose, PUN, IGF-1, NEFA, and insulin on day 0 did not differ between treatments ($P \geq 0.40$) but were included as covariates ($P \leq 0.02$) in the analyses of heifer BW and plasma concentrations of glucose, PUN, IGF-1, NEFA, and insulin, respectively. Liver concentrations of all trace minerals were not included as covariates ($P \geq 0.22$). Heifer overall average daily gain (ADG) and total DM intake of hay and supplement (day 0 to 45) were tested for fixed effects of treatment using pen(treatment) as random effect. Effects of block were included in all statistical analyses but removed from model if $P > 0.10$. All results are reported as least-squares means. Data were separated using PDIFF if a significant F-test was detected. Significance was set at $P \leq 0.05$, and tendencies were noted if $P > 0.05$ and ≤ 0.10.

RESULTS

Effects of day ($P < 0.0001$), but not treatment and treatment × day ($P \geq 0.28$), were detected for heifer BW. Effects of treatment tended to be detected ($P = 0.10$) for overall ADG from day 0 to 45, which was the least for TM heifers ($P \leq 0.05$) and did not differ among LMB, PSPF, and PS heifers ($P \geq 0.60$; Table 3).

Effects of treatment × week and treatment were detected for average daily supplement DMI ($P < 0.0001$), but not daily hay DMI ($P \geq 0.63$). From week 1 to 7, daily supplement DMI was always least for TM and greatest for PS heifers ($P \leq 0.05$) and did not differ ($P \geq 0.56$) between LMB and PSPF heifers (Figure 1). However, supplement DMI of PS heifers did not differ from week 1 to 7 ($P \geq 0.62$), whereas supplement DMI of TM, PSPF, and LMB tended to increase on week 7 vs. all previous weeks ($P \leq 0.10$; Figure 1). Mean daily hay DMI across treatments from day 0 to 45 was 4.04 ± 0.117 kg, whereas overall daily supplement DMI was least for TM and greatest for PS heifers ($P \leq 0.001$) and did not differ ($P = 0.84$) between LMB and PSPF heifers (60, 92, 91, and 386 ± 4.9 g/d for TM, LMB, PSPF, and PS heifers, respectively).

Effects of treatment were detected for total intake of supplement CP and TDN, and total intake of DM, CP, and TDN (supplement + hay) from day 0 to 45 ($P \leq 0.01$), but not for total intake of hay DM, CP, TDN, RDP, and RUP, and G:F from day 0 to 45 ($P \geq 0.52$; Table 4). Total intake of supplemental DM and TDN were least for TM and greatest for PS heifers ($P \leq 0.0005$) and did not differ ($P \geq 0.91$) between LMB and PSPF heifers (Table 4). Total intake of DM and TDN were least for TM heifers ($P \leq 0.03$) and did not differ ($P \geq 0.66$) among LMB, PSPF, and PS heifers (Table 4). Total supplemental intake of CP and RDP and total intake of CP and RDP (supplement + hay) were least for TM and greatest for PS heifers ($P \leq 0.05$) and did not differ ($P \geq 0.70$) between LMB and PSPF heifers (Table 4). Total intake of RUP was greatest for PS heifers ($P < 0.0001$) and did not

Table 3. Growth performance of Nellore heifers offered free choice access to warm-season grass hay and receiving trace mineral supplement (TM), sugarcane molasses cooked block (LMB), pair-fed protein supplement (PSPF), and a commercial protein supplement (PS) from day 0 to 45 ($n = 100$ heifers; five heifers/pen; five pens/treatment)[1]

	Treatment[1]					P-value	
Item	TM	LMB	PSPF	PS	SEM	Treatment × day	Treatment
BW[2,3], kg							
Day 24	186	188	188	188	0.95	0.60	0.28
Day 45	194	196	195	196	0.95		
ADG, kg/d							
Day 0 to 45	0.18[a]	0.26[b]	0.25[b]	0.27[b]	0.029	-	0.10

[a,b]Within a row, means without a common superscript differed ($P \leq 0.05$).

[1]TM = a complete vitamin/trace mineral mix supplement offered in a loose meal form (66.6% of LMB supplement DMI); LMB = free choice access to a low-moisture, cooked sugarcane molasses-based block; PSPF = protein supplement offered in a loose meal form and pair-fed to achieve similar supplement intake of DM, TDN, and CP of LMB heifers; PS = a commercial protein supplement offered in a loose meal form at 0.2% of BW (DM basis).

[2]Average of full BW collected on day −1 e 0 was included as covariate ($P < 0.0001$).

[3]Full BW on day 24 and 45 represent the average of full BW collected on day 24 and 25, and 45 and 46, respectively.
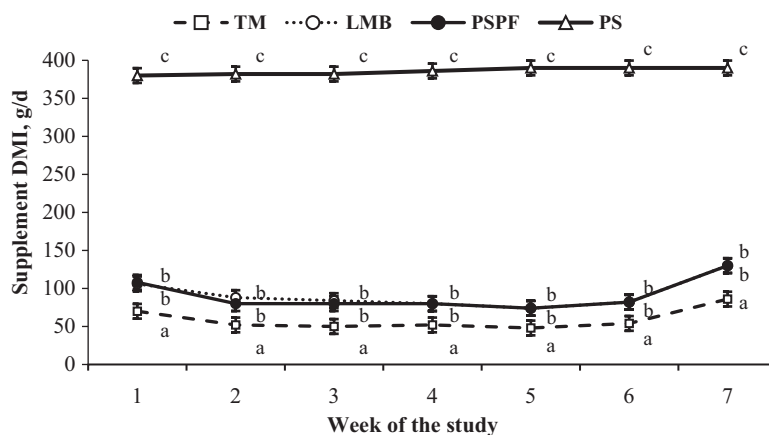
Figure 1. Daily supplement DMI from week 1 to 7 (day 0 to 45) of Nellore heifers offered free choice access to warm-season grass hay and receiving trace mineral supplement (TM), sugarcane molasses cooked block (LMB), pair-fed protein supplement (PSPF), and a commercial protein supplement (PS) from day 0 to 45 (n = 100 heifers; five heifers/pen; five pens/treatment). Effects treatment × week were detected for average daily supplement DMI ($P < 0.0001$). Supplement DMI of PS heifers did not differ from week 1 to 7 ($P \geq 0.62$), whereas supplement DMI of TM, PSPF, and LMB tended to increase on week 7 vs. all previous weeks ($P \leq 0.10$) [a-c]Within a week, means without a common superscript differed ($P \leq 0.05$).

differ ($P \geq 0.16$) among LMB, PSPF, and TM heifers (Table 4).

Plasma concentrations of glucose, PUN, IGF-1, NEFA, and insulin did not differ ($P \geq 0.57$) among treatments on day 0 but were included as covariates ($P \leq 0.02$) to adjust the plasma concentrations of glucose, PUN, IGF-1, NEFA, and insulin, respectively, obtained on day 24 and 45. Effects of treatment × day and treatment were not detected ($P \geq 0.61$) for plasma concentrations of IGF-1, insulin, and NEFA. Effects of treatment, but not treatment × day ($P \geq 0.12$), were detected for plasma concentrations of PUN ($P = 0.005$) and tended to be detected for plasma concentrations of glucose ($P = 0.08$), which were both least for TM heifers ($P \leq 0.03$) and did not differ ($P \geq 0.17$) among LMB, PSPF, and PS heifers (Table 5).

Effects of treatment were not detected ($P \geq 0.45$) for accumulated total intake of trace minerals from day 0 to 45 (Table 6). Liver concentrations of trace minerals were not included as covariates ($P \geq 0.22$) to adjust the liver concentrations of trace minerals on d 45. Effects of treatment and treatment × day were not detected ($P \geq 0.13$) for liver concentrations of trace minerals (Table 6).

DISCUSSION

Aubel et al. (2011) observed that LMB consumption of mature beef cows declined over time (0.30 to 0.12 kg/d) as the forage transitioned from winter dormancy to active spring growth. Similarly, Bailey et al. (2008) reported that LMB consumption of mature beef cows increased from 0.14 to 0.36 kg/d as forage chemical composition decreased. Heifers assigned to LMB supplementation did not receive

concentrate supplementation before the start of the study, and hence, the greater consumption of LMB supplement on week 1 likely reflects the adaptation period to LMB supplementation. After adaptation, LMB consumption remained constant and at the manufacturer recommendations until week 6. In contrast to studies described above, LMB consumption increased on week 7, despite the greater forage quality during the last 15 d of the study. The exact reasons for this response is unknown but it demonstrates that other factors beyond forage quality may impact LMB consumption, potentially rainfall, season, animal category, BW, and supplement composition. For instance, beef calves grazing bahiagrass pastures and fed trace mineral-fortified LMB had greater supplement DMI compared with non-fortified LMB calves (272 vs. 395 g/d, respectively; Ranches et al., 2018), whereas LMB supplement in Aubel et al. (2011) and Bailey et al. (2008) contained significantly less CP compared to the present study (4 and 30 vs. 46% CP, respectively).

Positive effects of LMB supplementation on intake of low-quality forages have been previously reported (Badurdeen et al., 1994; Greenwood et al., 1998, 2000). Badurdeen et al. (1994) observed a 10% increase in forage intake when bull calves were offered a 56% CP molasses-based LMB, and Greenwood et al. (1998) reported a 13% increase in forage intake when a 30% CP molasses-based LMB was provided. However, forage DMI did not differ among treatments in the present study. Moore et al. (1999) reported that supplements decreased voluntary forage intake when supplemental TDN intake was > 0.7% BW, when forage TDN:CP ratio was < 7 (adequate CP), or when voluntary forage intake was > 1.75% BW. Supplemental TDN

Table 4. Total nutrient intake and overall feed efficiency of Nellore heifers offered free choice access to warm-season grass hay and receiving trace mineral supplement (TM), sugarcane molasses cooked block (LMB), pair-fed protein supplement (PSPF), and a commercial protein supplement (PS) from day 0 to 45 (n = 100 heifers; five heifers/pen; five pens/treatment)[1]

Item	TM	LMB	PSPF	PS	SEM	P-value Treatment
Total intake day 0 to 45[2], kg						
DM						
Hay	182	180	184	191	5.7	0.61
Supplement	2.56[a]	3.94[b]	3.87[b]	17.4[c]	0.208	<0.0001
Total	185[a]	184[a]	188[a]	208[b]	5.8	0.04
TDN						
Hay	84.3	83.5	85.1	88.3	2.63	0.60
Supplement	0[a]	1.99[b]	1.97[b]	9.27[c]	0.156	<0.0001
Total	84.3[a]	85.4[a]	87.0[a]	97.6[b]	2.48	0.009
CP						
Hay	10.5	10.4	10.6	11.0	0.33	0.62
Supplement	0[a]	1.81[b]	1.79[b]	4.35[c]	0.145	<0.0001
Total	10.5[a]	12.2[b]	12.4[b]	14.4[c]	0.37	<0.0001
RDP						
Hay	8.9	8.9	9.0	9.4	0.28	0.59
Supplement	0[a]	1.69[b]	1.71[b]	3.64[c]	0.138	<0.0001
Total	8.9[a]	10.6[b]	10.7[b]	12.0[c]	0.33	<0.0001
RUP						
Hay	1.58	1.56	1.59	1.65	0.049	0.62
Supplement	0[a]	0.12[c]	0.08[b]	0.71[d]	0.009	<0.0001
Total	1.58[a]	1.69[a]	1.67[a]	2.37[b]	0.052	<0.0001
Overall G:F day 0 to 45[3]	0.049	0.063	0.058	0.055	0.007	0.52

[a–c]Within a row, means without a common superscript differed (P ≤ 0.05).

[1]TM = a complete vitamin/trace mineral mix supplement offered in a loose meal form (66.6% of LMB supplement DMI); LMB = free choice access to a low-moisture, cooked sugarcane molasses-based block; PSPF = protein supplement offered in a loose meal form and pair-fed to achieve similar supplement intake of DM, TDN, and CP of LMB heifers; PS = a commercial protein supplement offered in a loose meal form at 0.2% of BW (DM basis).

[2]Calculated using sum of the average intake of each respective nutrient from hay and supplement at each 15-d interval.

[3]Calculated by dividing total BW gain by total DMI from day 0 to 45.

intake ranged from 0.02 to 0.11% of BW and forage TDN:CP ratio was between 7.3 and 9.2, whereas TM heifers had a voluntary forage consumption of 4.04 kg/d, which represents 2.13% of the average BW from day 0 to 45. Depressions in neutral detergent fiber (NDF) digestion have been reported when sugarcane molasses was supplemented at levels of at least 15% of the dietary DM to cattle fed low-quality forage (Brown, 1993; Kalmbacher et al., 1995). In the present study, the contribution of sugarcane molasses was 1.1% of total diet DMI of LMB heifers. Consequently, the lack of treatment effects on forage DMI may not be attributed to sugar-induced depression in NDF digestion or to the supplementation levels utilized in the present study.

An increase on forage intake was expected as RDP supplementation generally improves utilization of low-quality warm-season forages (Köster et al., 1996). Daily RDP requirements for cattle fed low-quality forage are approximately 11% of TDN intake (Köster et al., 1996). Hence, TM heifers consumed slight less RDP than the daily requirement (197 vs. 206 g/d of RDP, respectively), which suggests that responses to supplementation may not have been maximized, and LMB, PSPF, and PS supplementation did not cause dramatic changes to RDP consumption and potentially forage digestion. According to NRC (2016) and using the observed hay and supplement DMI of each treatment, heifers required 188 g/d of metabolizable protein (MP) and 7.85 Mcal/d of metabolizable energy (ME) for an ADG of 0.1 kg/d. However, estimated MP consumption were 217, 262, 262, and 292 g/d, whereas ME consumption were 6.29, 7.83, 7.82, and 8.81 Mcal/d for TM, LMB, PSPF, and PS heifers, respectively. Therefore, protein consumption was not the limiting factor for any treatment group to experience the observed ADG in the present study. In addition, TM heifers were energy-deficient and consumed 1.56 Mcal/d less than their daily ME requirements, which explains the less ADG compared to all remaining treatments. Heifers assigned to PS treatment consumed an additional 1 Mcal/d of ME compared to LMB and PSPF, which perhaps was not sufficient to induce significant greater ADG in a 45-d feeding period. It is possible that differences in growth performance between LMB and PSPF vs. PS heifers would be observed with greater supplementation periods (perhaps >60 d).

Only plasma concentrations of glucose and PUN differed among treatments and were greater for LMB, PSPF, and PS vs. TM heifers, which reflect the differences on ADG and can be associated with differences in energy and protein intake among treatments. Insulin and IGF-1 synthesis is directly influenced by energy intake and circulating glucose concentrations (Vizcarra et al., 1998), whereas plasma concentrations of PUN are positively associated with intake of CP, RDP, and concentrations of ruminal ammonia (Hammond, 1997). Optimal PUN concentration in beef heifers ranges from 11 to 15 mg/dL (Byers and Moxon, 1980), indicating that all heifers in the present study consumed adequate amounts of CP and RDP, except for TM heifers which were slightly below the optimum PUN levels. Despite the greatest total intake of TDN

Table 5. Plasma concentrations of glucose, IGF-1, insulin, urea N (PUN), and nonesterified fatty acids (NEFA) of Nellore heifers offered free choice access to warm-season grass hay and receiving trace mineral supplement (TM), sugarcane molasses cooked block (LMB), pair-fed protein supplement (PSPF), and a commercial protein supplement (PS) from day 0 to 45 (n = 100 heifers; five heifers/pen; five pens/treatment)[1]

	Treatment[1]					P-value	
Plasma[2]	TM	LMB	PSPF	PS	SEM	Treatment × day	Treatment
Glucose, mg/dL	83.6[a]	90.9[b]	96.1[b]	95.5[b]	3.54	0.88	0.08
IGF-1, ng/mL	33.1	35.1	34.5	37.8	2.6	0.95	0.61
Insulin, μIU/mL	7.11	6.69	7.02	6.97	0.474	0.78	0.93
PUN, mg/dL	9.6[a]	11.1[b]	11.9[b]	11.1[b]	0.40	0.12	0.005
NEFA, mEq/L	0.148	0.153	0.173	0.154	0.023	0.87	0.78

[a,b]Within a row, means without a common superscript differed (P ≤ 0.05).

[1]TM = a complete vitamin/trace mineral mix supplement offered in a loose meal form (66.6% of LMB supplement DMI); LMB = free choice access to a low-moisture, cooked sugarcane molasses-based block; PSPF = protein supplement offered in a loose meal form and pair-fed to achieve similar supplement intake of DM, TDN, and CP of LMB heifers; PS = a commercial protein supplement offered in a loose meal form at 0.2% of BW (DM basis).

[2]Plasma concentrations of glucose, IGF-1, insulin, PUN, and NEFA were included as covariates (P ≤ 0.02). Means shown above represent the average plasma concentration of each respective parameter obtained on day 24 and 45.

and CP, plasma concentrations of glucose and IGF-1 of PS heifers were only numerically greater, whereas plasma concentrations of insulin and PUN did not differ compared with LMB heifers. Although plasma concentrations of NEFA did not differ among treatments, NEFA may increase the expression of gluconeogenic enzymes and decrease the uptake of glucose by body tissues (White et al., 2011), which may explain the numerical increase of plasma NEFA concentrations of LMB, PSPF, and PS vs. TM heifers. Blood samples were collected immediately before morning feeding at the time of BW collection in order to minimize gut fill effects on BW results and avoid disruption of diurnal feed intake. Thus, it is possible that the peak of release of all physiological parameters were missed. For instance, plasma concentrations of insulin generally peak between 1 and 2 h after feeding (Moriel et al., 2008).

Preweaning supplementation of mineral-fortified LMB is an efficient strategy to improve the trace mineral status of calves (Ranches et al., 2018). Beef calves grazing bahiagrass pastures and supplemented with trace mineral-fortified LMB had greater liver concentrations of Co, Cu, Mn, Se, and Zn compared with control nonfortified LMB calves, despite the less supplement DMI of fortified vs. nonfortified LMB calves (272 vs. 395 g/d, respectively; Ranches et al., 2018). Except for LMB, supplements offered in loose meal form were consumed entirely within 1 h after feeding. Although number of visits to LMB blocks were not measured in the present study, others have reported that cows spent more than 1 h per day visiting the sites where LMB was placed (Bailey and Welling, 2007; Bailey et al., 2008). Hence, it was expected that nutrient utilization

and trace mineral status would be enhanced in LMB heifers due to slower supplement consumption pattern compared to those offered supplements in a loose meal form. In the current study, TM and PSPF heifers were limit-fed their respective supplements to achieve similar trace mineral premix compared to LMB heifers and avoid confounding effects on trace mineral intake, which would allow the proper comparison of the impact of supplement delivery form on trace mineral status of heifers. As designed, total intake (hay + supplement) of trace mineral premix and each trace mineral element did not differ among treatments, but contrary to our hypothesis, liver concentrations of all trace minerals also did not differ among heifers. These results indicate that 1) heifers consumed adequate amounts of trace minerals and were not deficient in any trace mineral element, according to NRC (2005); and 2) bioavailability and/or absorption of trace minerals was likely not impacted by supplement delivery form. Similarly, Katulski et al. (2017) observed that tissue mineral content was proportionate to mineral intake of forage-fed heifers offered LMB or free choice mineral supplement, and that differences in mineral availability between loose mineral and LMB supplements were not evident.

Another potential partial explanation for the lack of treatment effects on liver trace mineral status is the impact of trace mineral antagonists (Arthington, 2017). Dietary S concentrations above 0.30% of DM may reduce Cu and Se bioavailability by associating with Mo in the rumen (Suttle, 1974; Mason, 1990; NRC, 2005). Estimated dietary S concentrations (hay + supplements) for all treatments were between 0.25% and 0.28%, which is slightly below the levels reported to induce Cu

Table 6. Total intake of premix and trace minerals from day 0 to 45 and liver concentrations of trace minerals of Nellore heifers offered free choice access to warm-season grass hay and receiving trace mineral supplement (TM), sugarcane molasses cooked block (LMB), pair-fed protein supplement (PSPF), and a commercial protein supplement (PS) from day 0 to 45 (n = 100 heifers; five heifers/pen; five pens/treatment)[1]

| | Treatment[1] | | | | | P-value | |
Item	TM	LMB	PSPF	PS	SEM	Treatment × day	Treatment
Total intake[2], day 0 to 45	---------------------------- g (DM basis) -----------------						
Trace mineral premix	95	98	96	106	13	-	0.92
Cu	2.58	2.60	2.61	2.83	0.122	-	0.45
Fe	55.9	55.6	56.4	59.3	1.75	-	0.47
Mn	3.12	3.13	3.15	3.35	1.08	-	0.42
Se	0.04	0.04	0.04	0.05	0.001	-	0.56
Zn	13.8	14.0	13.9	15.0	0.62	-	0.55
Liver concentration[3]	-------------------------- mg/kg (DM basis) -----------						
Cu	535	502	562	529	41	0.40	0.78
Fe	714	621	657	656	42	0.96	0.47
Mn	8.9	9.3	8.8	9.2	0.29	0.23	0.59
Mo	2.8	3.1	3.1	2.9	0.10	0.96	0.13
Se	0.59	0.64	0.61	0.65	0.036	0.13	0.61
Zn	166	171	170	188	11.4	0.55	0.49

[a,b]Within a row, means without a common superscript differed ($P \leq 0.05$).

[1]TM = a complete vitamin/trace mineral mix supplement offered in a loose meal form (66.6% of LMB supplement DMI); LMB = free choice access to a low-moisture, cooked sugarcane molasses-based block; PSPF = protein supplement offered in a loose meal form and pair-fed to achieve similar supplement intake of DM, TDN, and CP of LMB heifers; PS = a commercial protein supplement offered in a loose meal form at 0.2% of BW (DM basis).

[2]Calculated as the total supplement and hay DMI from day 0 to 45 multiplied by the respective average concentration of each trace mineral from supplement and hay obtained every 15-d period.

[3]Liver concentrations of all trace minerals were not included as covariates ($P \geq 0.22$). Means shown above represent the average liver concentration of the respective trace mineral obtained on day 0 and 45.

and Se deficiency. However, estimated dietary concentrations of Fe ranged from 285 to 302 mg/kg, which is within the dietary concentrations linked to Cu deficiency (250 to 500 mg of Fe/kg of diet DM; NRC, 2005). Iron is found in nearly all sources of cattle feed, water, and soil (Arthington, 2017), and hence, the relatively high levels of dietary Fe can be likely attributed to soil contamination of harvested forage offered to all heifers.

In conclusion, trace mineral-fortified LMB supplementation enhanced growth performance and had minor impact on physiological parameters of beef heifers fed low-quality warm-season forage compared with a nonprotein, trace mineral supplement offered in loose meal form. However, supplement delivery form (block vs. loose meal protein supplement) did not impact growth performance, physiological parameters, and liver trace mineral status when heifers experience similar but adequate intake of trace minerals. Hence, the use LMB supplements led to positive effects on growth without impacting trace mineral status compared to a loose meal trace mineral salt and led to similar growth performance and trace mineral status compared to a conventional

protein supplementation offered at 0.2% of body weight.

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